structure alignment of cofilin analogs that strongly supports our binding model. The implications of this binding model in the function and severing action of cofilin are discussed.

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Energy Coupling In Profilin-Dependent Actin Polymerization
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Profilin regulates actin polymerization in cells and is required for normal cell proliferation and differentiation. The molecular mechanism of profilin has been extensively studied and debated in the last 25 years, however no satisfactory explanation consistent with the laws of thermodynamics has yet been provided. In our recent article (Biophys. J. BioFAST: doi:10.1529/biophysj.108.134569) we demonstrate that the mechanism of profilin action could be based a general principle of indirect energy coupling which, as emerges from recent theoretical and experimental research, underlies many biologic processes.
In presence of profilin, there are two possible pathways for actin filament elongation. First pathway (g) is direct elongation through binding of G-actin subunits to the barbed end to obtain a filament one subunit longer. The second pathway (pg) is elongation through binding of profilin to G-actin, formation of profilin-actin complex, then binding of the profilin-actin complex to the barbed end with subsequent dissociation of profilin. In the absence of profilin, there is only one pathway g_0, which is the pathway g in the absence of profilin.
Our analysis shows that the pathway g for filament elongation in presence of profilin is not energetically equivalent to the pathway g_0 for the filament elongation in the absence of profilin due to the dependence of standard free energy change for both pathways g and pg on profilin concentration. Conventional calculations of energy imbalance have neglected the difference between the pathways g and g_0. We found that profilin can lower actin critical concentration even when the pathways g and pg are energetically equivalent. In this case the existence of the pathway pg can drive the filament nucleotide profile toward ATP-bound F-actin, making both pathways g and pg more energetically favorable than the single pathway g_0 available in the absence of profilin.

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Computational Study Of Viscoelasticity Of Crosslinked Actin-like Networks
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Mechanical force is very significantplays many important roles in eukaryotic cells in whichwhere the cross-linked actin cytoskeleton consisting largely of actin and actin binding proteins is one of the major structural components. Thus, the investigationit is critically crucial to study the of rheological properties of actin networks is indispensable in order to elucidate cell mechanics and various related cellular processes. Using Brownian dynamics, we develop a computational model equipped withthat incorporates virtues features such as including repulsive forces between actin filaments, realistic network morphology, and the consideration oftakes into account the stiffness, binding, and unbinding of actin cross-linking protein (ACP). Via bulk rheology and the analysis of thermal fluctuations of actin filaments, we elucidate investigate the viscoelastic properties of actin networks in under diverse aspectsconditions. We first validate ourThe model is first validated model by comparison with an experiments performed under similar conditions. Then, we study the influences of prestrain and ACP concentration on viscoelastic moduli, G' and G'', are examined. The storage modulus, G', tends is found to increase and becomes almost nearly independent of frequency at high ACP concentration or at large prestrain. We also find that the behavior of networks underAlso, under conditions of high prestrain, network rheology is governed by only a small portion of filaments that are highly stretched. Inclusion of ACP unbinding events under high prestrain results in stress relaxation and also leads to a power law behavior in G' as observed in many cells, and causes the loss modulus, G'', to increase at low frequency. We observe Nnonlinear stress-strain behaviors of actin networks are observed that are dependent on shear strain rates and the concentration and rupture of ACP. [Supported by GM076689]

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Weak to Strong Transition at the Actin-Myosin Interface Detected by Sensitized Emission Luminescence Resonance Energy Transfer (SELRET)
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Force is generated upon the transition of the actin-myosin complex from weak-to strong-binding states. To detect changes at the actin-myosin interface during this transition, we have detected sensitized emission luminescence resonance energy transfer (SELRET) from a luminescent Tb-chelate donor (attached to C374 of actin) to two kinds of acceptor-labeled myosin heads: (1) skeletal muscle myosin S1 labeled with tetramethylrhodamine iodoacetamide (TMRI) at C707 or (2) *Dictyostelium* myosin II motor domain (S1dC) labeled with iodoacetamidofluorescein (IAF) at a site engineered near the proposed binding interface (S619C). The emission of the Tb-donor, excited by a microsecond pulse, has an excited-state lifetime of 2 ms and is negligible at wavelengths where emission of acceptors is detected. Since the acceptors have ns excited-state lifetimes, the observed ms emission kinetics of acceptors is exclusively due to SELRET. Experiments on muscle S1 (labeled at C707) showed that the strong-to-weak transition results in a change of the distance from 5.5 nm (distribution width FWHM = 1.7 nm) to 6.0 nm (width 2.3 nm). The same transition with S1dC (labeled at C619) results a change of the distance from 4.0 nm (width 0.9 nm) to 4.2 nm (width 3.2 nm). Thus for both systems, the weak-to-strong transition results in a small change in the mean distance and a large decrease in the width of the distance distribution. These results add to the evidence that the weak-to-strong transition is fundamentally a disorder-to-order transition, whether it is measured within actin, within myosin, or at the interface between the two proteins.

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Crystal Structures of Monomeric Actin Bound to Cytochalasin D
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The fungal toxin cytochalasin D (CD) interferes with normal actin cytoskeletal dynamics by binding to the barbed end of actin filaments. Despite being extensively used as a tool for studying actin-mediated processes, the exact location and nature of its binding to actin are unknown. We have determined two crystal structures of a cytoplasmic actin, engineered to remain monomeric, with CD. One was obtained by soaking actin crystals with CD, and the other by co-crystallization. The CD-binding site, in the hydrophobic cleft between actin sub-domains 1 and 3, is identical in the two structures. Polar and hydrophobic contacts play equally important roles in CD binding, with six hydrogen bonds stabilizing the actin-CD complex. While many actin-binding proteins and marine toxins target this cleft, they primarily target the front half of this cleft (viewing actin with sub-domain 2 on the upper right). CD differs in that it targets the back half of this cleft. Our analysis suggests that contacts with this region of the cleft would ensure filament capping without severing. Importantly, the actin molecule in the co-crystallized actin-CD structure shows novel conformational changes in response to ligand binding. These include an ~6° inter-domain rotation, causing small changes in crystal packing that enables the ordering of the D-loop (DNase I-binding loop), which is disordered in most structures of actin. The D-loop adopts an extended, non-periodic conformation and is stabilized by contacts with neighboring actin monomers. Based on the shift in position of a putative nucleophilic water, we postulate a mechanism for CD-induced enhancement of actin-catalyzed ATP hydrolysis. We speculate that these changes represent a potential conformation that the actin monomer can adopt on the pathway to polymerization or in a filament.

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Structure of an Actin Trimer Stabilized by a Tandem W Domain Hybrid Construct
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The study of the actin filament is one of the major problems of structural biology. Uncontrollable polymerization has interfered with our ability to obtain crystals of the filament. Proteins that initiate actin polymerization in cells have the natural ability to stabilize multiple actin subunits into a filament-like conformation, allowing them to overcome the rate-limiting step in polymerization, i.e. the formation of actin dimers and trimers. With the exception of formins, known filament nucleators use the Wiskott-Aldrich syndrome protein (WASP) homology 2 (WH2 or W) domain for interaction with actin. The W domain is a short (17–27 aa) actin-binding motif. A common architecture, found in filament nucleators such as Spire, Cobl, VopL, and VopF, consists of tandem W domains that tie together three to four actin monomers to form a polymerization nucleus. We have engineered a stable actin trimer stabilized by a tandem W domain hybrid construct that also includes filament barbed and pointed end capping elements. The structure of the actin trimer was first studied in solution using x-ray scattering. Different crystal forms of the trimer have been obtained. We will present these studies. In particular, we will show how tandem W domains stabilize a polymerization nucleus by organizing actin subunits into a filament-like conformation.
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